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F_2-isoprostane and prostaglandin $F_{2\alpha}$ metabolite excretion rate and day to day variation in healthy humans

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Summary Isoprostanes are mainly formed in vivo by a non-enzymatic free radical catalysed oxidation of arachidonic acid. Studies have indicated that a major isoprostane, 8-iso-$PGF_{2\alpha}$ in plasma and urine is a reliable biomarker of oxidative stress. Prostaglandins are formed by enzymatic oxidation of arachidonic acid catalysed by cyclooxygenase (COX). 15-Keto-dihydro-$PGF_{2\alpha}$, a major metabolite of prostaglandin $F_{2\alpha}$ in plasma, and also found in urine, is considered to be a useful biomarker of inflammation. To investigate the excretion pattern and day to day variation of 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ in healthy individuals, morning urine samples were collected from 13 volunteers on 10 successive days. The samples were analysed for free 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ by radioimmunoassay. The mean excretion rate of 8-iso-$PGF_{2\alpha}$ was 0.27 ± 0.11 nmol/mmol creatinine (mean $\pm$ SD, $n=13$) and the coefficient of variation was 42% during the 10 days. The mean excretion rate of 15-keto-dihydro-$PGF_{2\alpha}$ was 0.46 ± 0.19 nmol/mmol creatinine, giving a coefficient of variation of 41%. The mean values of 8-iso-$PGF_{2\alpha}$ were significantly correlated with the mean values of 15-keto-dihydro-$PGF_{2\alpha}$ ($r=0.68$, $P=0.01$). In conclusion, day to day biological variation in urinary excretion rate of 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ should be taken into account in evaluating a clinical study unless a large increase or decrease of these parameters has been obtained. © 2001 Harcourt Publishers Ltd

INTRODUCTION

Oxidative stress has been implicated in the pathophysiology of several human diseases including atherosclerosis, complications of diabetes, adult respiratory distress syndrome, cancer, Alzheimer's disease and in the process of ageing.[1–4] Isoprostanes belong to a family of prostaglandin derivatives, which are mainly formed by free radical peroxidation of arachidonic acid and have been considered to reflect oxidative stress in vivo.[5] One of the major isoprostanes, 8-iso-$PGF_{2\alpha}$, is relatively chemically stable and can be detected both in plasma and urine in animals as well as in humans and is considered to be a reliable biomarker of oxidative stress. Levels of 8-iso-$PGF_{2\alpha}$ in plasma or urine have been found to be elevated in conditions associated with oxidative stress like various rheumatic diseases[6] and conditions considered as cardiovascular risk factors including diabetes mellitus,[7] cigarette smoking,[8,9] and hypercholesterolaemia.[10] Other reported associations with enhanced formation of F_2-isoprostanes are high alcohol consumption,[11] endotoxaemia[12] and experimental hepatotoxicity.[13,14] 8-Iso-$PGF_{2\alpha}$ is also considered to possess biological activity in forms of kidney vasoconstriction[15] and pulmonary artery dilatation[16] in animal studies. The isoform of cyclooxygenase (COX-2) is induced and expressed in different cell types during inflammatory processes catalysing the formation of prostaglandins and related compounds enzymatically from arachidonic acid. 15-Keto-dihydro-$PGF_{2\alpha}$ is a major metabolite of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in plasma and is shown to be a useful indicator of inflammation.[17]

By measuring both 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ in plasma and/or urine a possible connection between oxidative stress and inflammation has been observed in experimental studies of acute endotoxaemia

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(septic shock model) in pigs, where the induced inflammation was accompanied by massive oxidative stress[12] and in experimental hepatotoxicity in rats where the induced oxidative stress was observed with a pronounced inflammatory response.[13] There is evidence for involvement of both inflammation and oxidative stress in initiation and progress of arteriosclerotic cardiovascular disease in humans[18,19] and also in ischaemia/reperfusion injury cases[20] which suggests a possible linkage of oxidative stress and inflammation through free radical and cyclooxygenase pathways.

It is also known that 8-iso-$PGF_{2\alpha}$ metabolizes rapidly in the body and a large quantity of this compound is excreted from the circulation into the urine within a few minutes. 15-Keto-dihydro-$PGF_{2\alpha}$ also undergoes rapid degradation mainly to various β-oxidised products but a substantial amount of this compound is excreted into the urine after the release of $PGF_{2\alpha}$.[21] Since both 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ are found in much higher quantity in the urine than in plasma it is adequate to measure these compounds in urine unless a frequent blood sampling regime is followed for plasma measurement.[17,22,23] A recent study with 10 healthy humans suggested that the urine for analysis of 8-iso-$PGF_{2\alpha}$ can be collected at any time of the day and that one single urine sample, preferably the morning sample, reflects the status of oxidative stress just as adequately as a 24-h urine collection when comparing the mean values of the 10 subjects.[24] Although 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ are considered to be reliable parameters of oxidative injury and inflammation, respectively, very little is known about their excretion pattern between days. It is therefore necessary to extend our knowledge about biological variation of these biomarkers in healthy individuals. Thus, the aim of this study was to investigate the day to day variation in the excretion rate of F_2-isoprostanes and a $PGF_{2\alpha}$ metabolite by measuring 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ in morning urine collected during 10 successive days in healthy humans.

MATERIALS AND METHODS

Sample collection

Urinary samples were collected from 13 healthy male and female volunteers (age 22–59 years), who had not taken any medication, on 10 successive days. All samples were collected in the morning (representative for about 6–8 h) and stored frozen at −70°C until analysis.

Chemicals

Unlabelled 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ were purchased from Cayman Chemicals (Ann Arbour, MI, USA). Tris-HCl, tris-base, EDTA-disodium salt and bovine-γ-globulin were purchased from Sigma Chemicals (St Louis, MO, USA); instagel scintillation cocktail from Packard Instruments Company (Meriden, CT, USA); and polyethylene glycol (MW 4000) from Merck (Germany). Unlabelled 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ standards, tritium labelled tracer and working antibody dilution were prepared in the RIA buffer. The tritium labelled 15-keto-dihydro-$PGF_{2\alpha}$ (specific activity: 6.77 TBq/mmol) was obtained from Amersham (Buckinghamshire, UK). Antibodies against both 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ were raised at our laboratory and well characterized.[17,23]

Radioimmunoassay of urinary 8-iso-$PGF_{2\alpha}$

The urinary samples (50 µl) were analysed for free 8-iso-$PGF_{2\alpha}$ without any prior extraction or purification by a newly developed radioimmunoassay.[23] In brief, an antibody was raised in rabbits by immunisation with 8-iso-$PGF_{2\alpha}$ coupled to BSA at the carboxylic acid by 1,1'-carbonyldiimmidazole method. The cross-reactivity of the antibody with 8-iso-15-keto-13,14-dihydro-$PGF_{2\alpha}$, 8-iso-$PGF_{2\beta}$, $PGF_{2\alpha}$, 15-keto-$PGF_{2\alpha}$, 15-keto-13,14-dihydro-$PGF_{2\alpha}$, TXB_2, 11β-$PGF_{2\alpha}$, 9β-$PGF_{2\alpha}$ and 8-iso-$PGF_{3\alpha}$ was 1.7, 9.8, 1.1, 0.01, 0.01, 0.1, 0.03, 1.8 and 0.6% respectively. The detection limit of the assay was about 23 pmol/L.

Radioimmunoassay of urinary 15-keto-dihydro-$PGF_{2\alpha}$

The urinary samples (50 µl) were analysed for 15-keto-dihydro-$PGF_{2\alpha}$ by a newly developed radioimmunoassay at our laboratory as described elsewhere.[17] In brief, an antibody was raised in rabbits by immunization with 15-keto-dihydro-$PGF_{2\alpha}$ coupled to BSA at the carboxylic acid by 1,1'-carbonyldiimmidazole method. The cross-reactivity of the antibody with $PGF_{2\alpha}$, 15-keto-$PGF_{2\alpha}$, PGE_2, 15-keto-13,14-dihydro-PGE_2, 8-iso-15-keto-13,14-dihydro-$PGF_{2\alpha}$, 11β-$PGF_{2\alpha}$, 9β-$PGF_{2\alpha}$, TXB_2, 8-iso-$PGF_{3\alpha}$ was 0.02, 0.43, <0.001, 0.5, 1.7, <0.001, <0.001, <0.001 and 0.01% respectively. The detection limit was about 45 pmol/L.

Urine creatinine assay

Creatinine concentration was determined in each urine sample by a colorimetric method using IL Test creatinine 181672-00 in a Monarch® 2000 centrifugal analyser (Instrumentation Laboratories, Lexington, MA, USA). The levels of 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ in urine were corrected for creatinine values.

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Statistics

The data are expressed as mean ± standard deviation (SD). Pooled SD were used in the calculations of the coefficients of variation (CV). The Spearman rank correlation was used for calculation of the correlation coefficient of the mean values. All calculations were performed using the statistical software package JMP (SAS Institute, Cary, NC).

RESULTS

Mean values for 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ excretion rate on 10 successive days in each subject, corrected for urinary creatinine values, are given in Table 1. The mean excretion rate of 8-iso-$PGF_{2\alpha}$ was 0.27 ± 0.11 nmol/mmol creatinine (mean ± SD, n=13) giving a coefficient of variation of 42%. The mean excretion rate of 15-keto-dihydro-$PGF_{2\alpha}$ was 0.46 ± 0.19 nmol/mmol creatinine (n=13) corresponding to a coefficient of variation of 41%. The mean values of 8-iso-$PGF_{2\alpha}$ were correlated with the mean values of 15-keto-dihydro-$PGF_{2\alpha}$ (r=0.68, P=0.01).

DISCUSSION

This study of healthy humans shows that a day to day variation (during 10 successive days) in urinary levels of 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ is about 42% and 41%, respectively. This variation includes both a biological variation and an intra-assay variation of the assayed samples. The variation due to the analysis technique used in this study has a coefficient of variation of about 12–15%[17,23] and the remaining observed variation (~26–30%) is therefore probably due to an individual variation in formation and excretion of the compounds between days. It is known that diet does not confound with isoprostane levels in humans;[25] thus, the influence of diet in this respect is not a variable factor. However, there are several factors that might cause the observed day to day biological variations of 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$. Most of the prostaglandins including 8-iso-$PGF_{2\alpha}$ have a very short half-life[22,26] and the metabolism and excretion kinetics may vary among different days and individuals. This is mainly due to differences in the formation and metabolism of these compounds depending on various endogenous regulating factors, i.e. enzymes and beta oxidation systems. Other reasons could be variability of endogenous defence mechanisms against oxidative stress and inflammation. This observation is also of importance in regard to the basal levels of other prostaglandin-like compounds which show a similar phenomenon.

The mean levels of urinary 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ in each person in our study correlate well with each other indicating a possible connection between the oxidative stress parameter and inflammatory response in healthy humans in basal conditions. However, the biosynthesis and kinetics of release patterns of these compounds are shown to be different in induced inflammation and oxidative stress conditions in experimental studies.[12,13] This might also vary with disease status due to its duration and disease flare.[6]

The results in this study show a mean level of urinary 8-iso-$PGF_{2\alpha}$ in healthy individuals at about the same level as seen in earlier studies where the same radioimmunoassay technique was used.[24] Subject No. 11 had a higher excretion of 8-iso-$PGF_{2\alpha}$ than the rest of the subjects. This could possibly be due to daily intake of iron supplementation in this subject. In vitro experiments have indicated that iron ions added to biological membrane systems increase the lipid peroxidation by the ions' property of easily donating or receiving a hydrogen atom.[27]

The precise role of free radical induced lipid peroxidation and enzymatic lipid peroxidation and the possible connections to human diseases are not yet thoroughly evaluated, partly owing to the unavailability of reliable analytical methods.[27] These highly specific radioimmunoassays for quantification of 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ offer chances to analyse human material and to determine lipid peroxidation in both basal and pathophysiological states. The high coefficients of variation in the urinary excretion of these biomarkers indicate that it is favourable to include a higher number of subjects when moderate levels of oxidative stress and inflammation are expected. If only a smaller number of subjects are available the urine samples should preferably be collected from more than one day per subject to circumvent the day to day variation of these eicosanoids.

Table 1 Mean levels of 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ in urine for 10 successive days in the 13 healthy subjects. The values are corrected for creatinine concentration

Subject No.	8-iso-$PGF_{2\alpha}$ (nmol/mmol creatinine)	15-keto-dihydro-$PGF_{2\alpha}$ (nmol/mmol creatinine)
1	0.26 ± 0.27	0.75 ± 0.39
2	0.25 ± 0.14	0.47 ± 0.24
3	0.11 ± 0.03	0.24 ± 0.07
4	0.23 ± 0.05	0.47 ± 0.16
5	0.19 ± 0.05	0.36 ± 0.13
6	0.32 ± 0.09	0.38 ± 0.14
7	0.34 ± 0.06	0.44 ± 0.11
8	0.20 ± 0.06	0.42 ± 0.10
9	0.21 ± 0.05	0.23 ± 0.12
10	0.37 ± 0.11	0.71 ± 0.16
11	0.62 ± 0.17	0.66 ± 0.19
12	0.20 ± 0.10	0.39 ± 0.21
13	0.22 ± 0.06	0.50 ± 0.19
All	0.27 ± 0.11	0.46 ± 0.19

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In conclusion, day to day variation of urinary levels of 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ should be taken into account in the evaluation of a clinical study if a small number of urinary samples are collected unless a higher increase or decrease of these parameters are obtained in the experiment.

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